SOME MYCOLOGICAL ASPECTS OF THE BIOLOGY OF MYCORRHIZA IN THE ERICACEAE

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INTRODUCTION

Members of the Ericaceae are normally infected by a mycorrhizal fungus. Two types of mycorrhizal association are found, known as the 'ericoid' and 'arbutoid' types. The ericoid type is the most important and is found in such genera as *Erica*, *Vaccinium*, *Rhododendron* and *Calluna* where infection typically consists of intracellular hyphal complexes in the outer cortical cells of the lateral 'hair' roots. The arbutoid mycorrhizas are of the ect-endo type and are found in the genera *Arctous*, *Arbutus*, and *Arctostaphylos*.

Routine methods have recently been developed for the isolation and culture of the endophyte of ericoid mycorrhizas (Pearson & Read, 1973). The view expressed repeatedly by Rayner (1913, 1922) that species of *Phoma* were the mycorrhizal fungi of Ericaceae and that the infection was systemic has been discounted (Harley, 1969; Pearson & Read 1973). One true mycorrhizal isolate has now been induced to fruit in pure culture and has been named *Pezizella ericae* sp. nov. (Read, 1974).

Since the long controversy surrounding the identity of the endophyte has been resolved interest now centres upon the biological importance of ericoid

mycorrhizas. Studies both of the extent of infection in individual plants and of the physiological role of mycorrhiza are required.

This paper presents an analysis of the distribution and anatomy of ericoid mycorrhizal infection in *Calluna vulgaris* and contrasts the pattern of mycorrhizal infection with that found in plants with vesicular-arbuscular infection. The second paper (Stribley & Read, this volume) presents some results of investigations into the physiological relationship between host and endophyte.

MATERIALS AND METHODS

Turves containing *Calluna vulgaris* seedlings of ages ranging from approximately 2 months to one year were collected from heathland sites at Beeley Moor in Derbyshire (Nat. Grid Ref. SK292664 and SK293668). In the laboratory the seedlings were carefully teased out of the soil in order to retain the fine hair roots. Camera lucida drawings were made of 6 typical seedlings in which the entire root system had been successfully extracted. The major dimensions of the root systems and of the cortical cells were measured, before the entire root systems were excised and stained in cotton blue and lactophenol. The staining enabled the distribution of mycorrhizal infection to be mapped. Representative parts of the root systems were then mounted in methyl cellulose and serial transverse sections were cut in a cryostat.

By comparing the results of the mapping and sectioning techniques it was possible to categorise the various levels of intensity of mycorrhizal infection in each seedling. The proportion of the root in which 98 to 100% of the cells were mycorrhizal was first estimated. This portion was termed the fully mycorrhizal category (Category 1). The proportions falling into 5 lower categories of infection was then estimated viz 75 to 98% infection (Category 2), 50 to

75% (Category 3), 25 to 50% (Category 4), 0 to 25% (Category 5) and no infection (Category 6).

The figures obtained from the observations were used in conjunction with the microscopical analysis to estimate the total volume of the root system occupied by fungal tissue and the total number of fungal entry points per unit root length and per plant.

RESULTS

Figures for total root lengths and for the proportions of each root system falling into the various categories are presented in Table 1. The mean figure of the extent of mycorrhizal infection in the seedlings is 65%, and of this clearly the largest proportion falls into the categories representing above 75% intensity. The categories with lower levels of infection represent only a small proportion (14%) of the total root length. As revealed in Fig. 1, the main sites of heavy infection are the lateral 'hair' roots where fungal penetration begins about 0.5 mm behind the root cap and extends backward into older parts of the system. In these 'hair' roots the great majority of the outer cortical cells become infected. Figs. 2a and b show a typical hair root of Category 1 infection intensity in surface view and in transverse section. Transverse sections reveal the great density of fungal material in the cortical cells and the high proportion of the total volume of root occupied by fungus in Category 1 infection zones. Such sections (Fig. 2b) reveal that up to 80% of the total root volume is occupied by fungal material. If the volume occupied by fungus in 100% infected root is 80% then by making proportionate reductions in the other categories and taking into account the proportion of the total root length which each category occupies it is possible

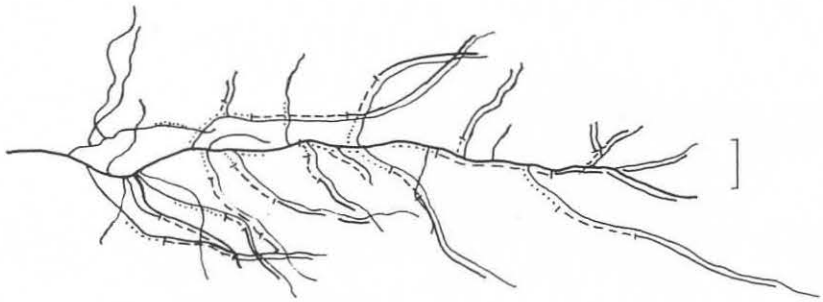

Fig. 1. Camera lucida drawing of the root system of seedling 1. Above each infected part of the root system the category of infection is indicated thus:- _____ Cat.1; ------ Cat. 2; ..... Cats. 3 to 5. For explanation of infection categories see text. Scale line is 1cm.

to calculate the volume of the total root system occupied by the endophyte. The results of such calculations (Table 2) indicate that in the six seedlings for which detailed measurements have been made 42.4% of the total root volume is occupied by fungal material.

Careful examination of individual infected cortical cells normally reveals at least one fungal entry point per cell, and a larger number is normal (Fig. 3a and b). As many as twelve entry points have been observed per cell. By measuring the length and diameter of roots and individual cortical cells in the various categories it is possible to estimate the number of entry points in any length of root.

Table 1. The total root lengths of six representative seedlings of *Calluna vulgaris*, and details of the extent of their mycorrhizal infection.

Seedling No.	Total root length cm	Proportion of root that is mycorrhizal %	% length of root in each infection category*					
			1	2	3	4	5	6
1	88.4	70	34	18	11	5	2	30
2	140.1	68	26	24	9	5	4	32
3	153.6	64	29	20	9	3	3	36
4	99.7	71	37	16	12	4	2	29
5	185.0	63	24	24	9	4	2	37
6	161.1	59	22	20	6	6	5	41
Mean	137.9	65	28	20	9	4	4	35

* For explanation of categories 1-6, see text.

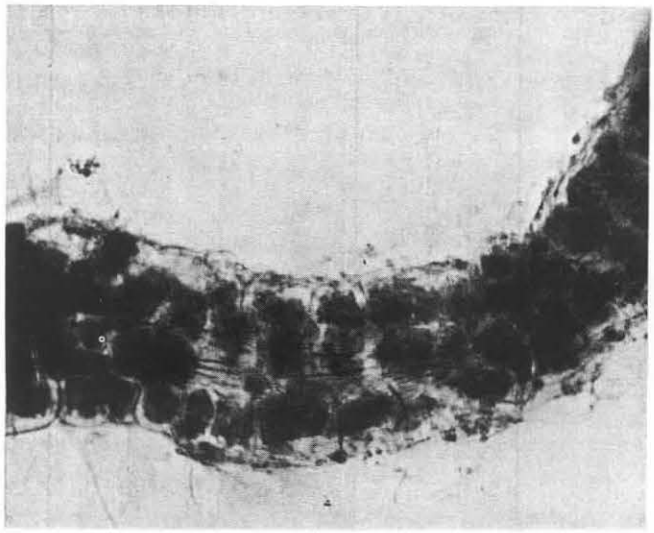

Fig. 2. a) Surface view of Category one root of *Calluna vulgaris* seedling

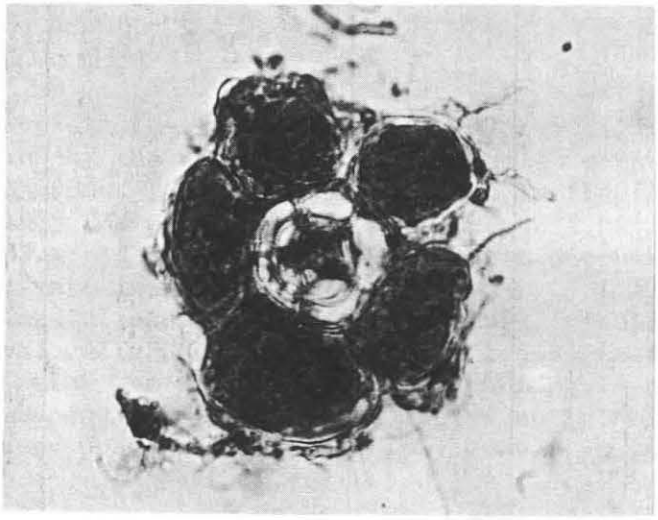

Fig. 2. b) Transverse section of a category one root showing the intensive infection in all the cortical cells.

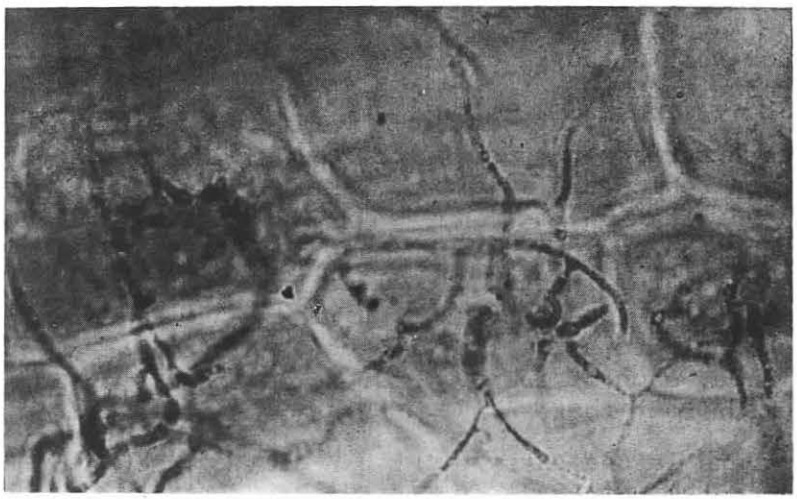

Fig. 3. a) Surface view of root showing numerous appressoria associated with entry points in cells of the Category one zone.

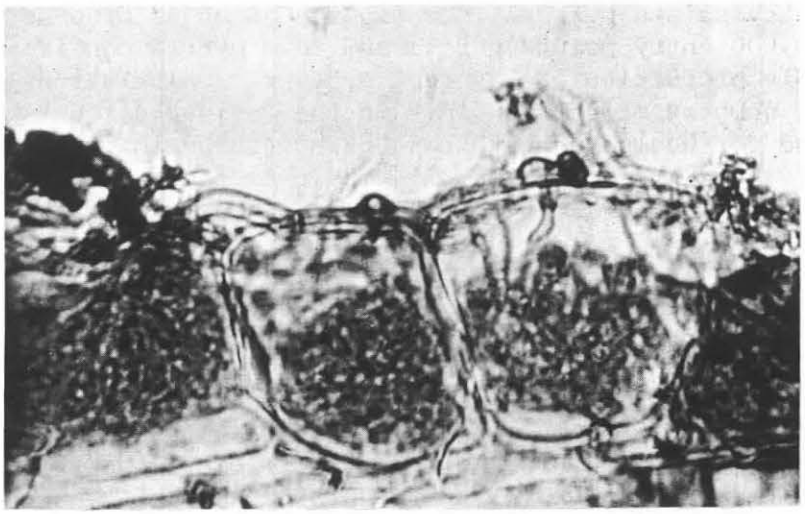

Fig. 3. b) Lateral view of cortical cells showing four entry points in one field of focus.

The diameter of typical hair roots of the seedlings was in the range of 40-60 μm, and the circumference was in the range 124-188 μm. The size and shape of individual cells is variable. Cells of square surface dimensions of 20-30 x 20-30 μm are common behind the root tip. In this region of very heavy infection there may be from 5 to 10 cells around the periphery of the root. Further back along the root cortical cells often become rectangular with a surface dimension of 25-45 x 15-20 μm. Assuming that there are five cells around the circumference, each of 25 x 25 μm outer dimension, then in 1 cm of root there are $5 \times \frac{10,000}{25} = 2000$ cells. If there is one entry point per cell, a figure of 2000 entry points per cm of root is obtained for Category 1 parts of the root. This figure is a very conservative one based on the assumption that there are only 5 cells around the circumference of the root and only one entry point per cell. Since there may be up to 10 cells around the circumference and 12 entry points per cell the figure can be as high as 48,000 entry points per cm and this over a considerable proportion of the root system. Calculations of numbers of entry points in the various categories and for whole root systems are presented in Table 3.

DISCUSSION

It is important to consider the extent to which the mycorrhizal status of the seedlings is comparable with that of mature plants of *Calluna vulgaris* and of other genera with ericoid mycorrhizas. Unfortunately it is virtually impossible to recover entire root systems of mature plants from the field. The fine roots of Ericaceae are particularly susceptible to breakage and any attempt to assess distribution of infection of mature plants will inevitably lead to gross errors. It is for this reason that field

Table 2. The calculated mean amounts of fungal tissue in each category of fungal infection and in the whole root system.

	Infection Category				
	1	2	3	4	5
Proportion of root falling into the category, mean of 6 seedlings, %.	28	20	9	4	4
Mean level of infection assumed for category, %.	100	87.5	62.5	37.5	12.5
Calculated volume occupied by fungus, %.	80	70	50	30	10
Calculated volume as proportion of whole root system, %.	22.4	14.0	4.5	1.2	0.3
Mean total volume occupied by fungus (6 seedlings), %.			42.4%		

Table 3. Calculated number of entry points in the various categories and in the entire root system.

	Infection Category				
	1	2	3	4	5
Number of entry points per cm	2000	1750	1250	750	250
Root length per category, cm	28	20	9	4	4
Number of entry points per category	56×10^3	35×10^3	11×10^3	3×10^3	1×10^3
Mean total number of entry points for seedlings assuming					
a) 1 entry point per cell			106×10^3		
b) 6 entry points			636×10^3		
c) 12 entry points			127×10^4		

seedlings were used in this study. Observations using older pot-grown plants from which most of the roots can be recovered suggest that the proportion of fine roots infected by endophyte is similar - about 60%. More of the infection is in the categories of lower infection intensity however.

The basic pattern revealed in *Calluna* is repeated in other ericaceous genera which have been examined. In *Vaccinium* and *Rhododendron*, however, the 'hair' roots are often of more robust dimensions with as many as 4 layers of cortical cells around the stele and a circumference made up of 20 or more cells.

When ericoid infections are compared with that found in other types of mycorrhiza, the major difference clearly lies in the amount of fungal tissue involved. In vesicular-arbuscular mycorrhizas reports of numbers of entry points vary from 2 to 20 per mm in strawberry (Mosse, 1959) to 6 per cm in onion (Sanders & Tinker, 1973). Observations in this laboratory indicate that the number in *Festuca ovina* may be up to 15 per cm. This compares with well over 1,000 per cm over much of the *Calluna* root.

Within the root, despite the general lack of inter-cellular ramification in ericaceous plants the fungus forms a significant proportion of the total tissue mass in most of the infected areas. This situation is in marked contrast with that in vesicular-arbuscular mycorrhizas in which, despite considerable internal ramifications, the ratio of fungal to higher plant tissue is always small. In transverse sections of roots infected with *Endogone* it is often difficult to detect fungal material.

In ecto-mycorrhizal species it has been estimated that 39% of the dry weight of the mycorrhizal root may be attributable to fungal tissue (Harley, 1969). It is clearly not possible to make direct measurements of the weight of endo-mycorrhizal fungi but some comparison may be drawn from observations of relative tissue proportions. In heavily mycorrhizal regions of *Calluna* roots it may be assumed that since

80% of the total volume of the root is occupied by fungal tissue a very high proportion of the weight of that tissue is attributable to the fungus. The proportion may be at least 80% since the fungal tissue is densely cytoplasmic relative to the neighboring vacuolate uninfected cells of the root.

It is difficult to envisage any living host tissue being unaffected physiologically by the penetration of such large masses of alien tissue. Indeed the invasion is so great that the survival and apparently healthy growth of the host is in many ways surprising. The major emphasis of research in this field should now be to investigate the basic physiology of the host-fungus relationship, with a view to elucidating the extent to which the association is truly mutualistic.

REFERENCES

HARLEY, J. L. (1969). *The biology of mycorrhiza*. Leonard Hill, London.

MOSSE, B. (1959). The regular germination of resting spores and some observations on the growth requirements of *Endogone* species causing vesicular-arbuscular mycorrhizas. *Trans. Br. mycol. Soc.*, 42, 274-286.

PEARSON, V. & READ, D. J. (1973). The biology of mycorrhiza in the Ericaceae. I. The isolation of the endophyte and synthesis of mycorrhiza in aseptic culture. *New Phytol.*, 72, 371-379.

RAYNER, M. C. (1913). The ecology of *Calluna vulgaris* L., *New Phytol.*, 12, 59-77.

RAYNER, M. C. (1922). Mycorrhiza in the Ericaceae. *Trans. Br. mycol. Soc.*, 8, 61-66.

READ, D. J. (1974). *Pezizella ericae* sp. nov. the perfect state of a typical mycorrhizal endophyte of Ericaceae. *Trans. Br. mycol. Soc.*, 63, 381-383.

SANDERS, F. E. & TINKER, P. B. (1973). Phosphate flow into mycorrhizal roots. *Pestic. Sci.*, 4, 385-395.